

5016 US Saxena 17 Jul 2003  
SEQUENCE LISTING

<110> Saxena, Shailendra K.  
<120> RIBONUCLEASES AND METHODS OF MAKING THEM  
<130> 5016 US  
<160> 74  
<170> PatentIn version 3.1  
<210> 1  
<211> 114  
<212> PRT  
<213> Artificial  
<220>  
<223> Recombinantly produced 2325p4 protein occurring naturally in rana pipiens eggs and embryos.  
<400> 1

Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile  
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp  
20 25 30

Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile  
35 40 45

His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly  
50 55 60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys Lys  
65 70 75 80

Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile  
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly  
100 105 110

Lys Cys

<210> 2  
<211> 342  
<212> DNA  
<213> Artificial  
<220>  
<223> 2325p4 DNA occurring naturally in rana pipiens eggs and embryos.

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<400> 2  
aaaccgaaag aagaccgtga atggaaaaaa ttcaaaaacta aacatatcac ttctcagtct 60  
gttgctgact tcaactgcaa ccgtactatg aacgaccgg cttacactcc ggacggtcag 120  
tgcaaaccga tcaacacttt catccattct actactggtc cggttaaaga aatctgccgt 180  
cgtgctactg gtcgtgttaa caaatcttct actcagcagt tcactctgac tacttgcaaa 240  
aacccgatcc gttgcaaata ctctcagtct aacactacta acttcatctg catcaacttgc 300  
cgtgacaact acccggttca tttcgtaaaa actggtaaat gc 342

<210> 3  
<211> 56  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:3 Contains XbaI restriction site.

<400> 3  
taattttgtt taactttaag aaggagatat accatgaaac cgaaagaaga ccgtga 56

<210> 4  
<211> 63  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:4 Complementary to SEQ ID NO:3

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ttcccattca cggtcttctt tcggtttcat ggtatatctc cttcttaaag ttaaacaaaa 60  
tta 63

<210> 5  
<211> 57  
<212> DNA  
<213> Artificial

<220>  
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<210> 6  
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<213> Artificial

<220>  
<223> SEQ ID NO:6 Complementary to SEQ ID NO:5

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<210> 7  
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<220>  
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<210> 8  
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<213> Artificial

<220>  
<223> SEQ ID NO:8 Complementary to SEQ ID NO:7

<400> 8  
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<210> 9  
<211> 52  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:9

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tttcatccat tctactactg gtccggtaa agaaatctgc cgtcgtgcta ct 52

<210> 10  
<211> 52  
<212> DNA  
<213> Artificial

<220>  
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<400> 10  
cacgaccagt agcacgacgg cagatttctt taaccggacc agtagtagaa tg 52

<210> 11  
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<212> DNA  
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<220>  
<223> SEQ ID NO:11

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<210> 12  
<211> 54

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<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:12 Complementary to SEQ ID NO:11

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54

<210> 13  
<211> 57  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:13

<400> 13  
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57

<210> 14  
<211> 57  
<212> DNA  
<213> Artificial

<220>  
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<400> 14  
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57

<210> 15  
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<213> Artificial

<220>  
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<400> 15  
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60

<210> 16  
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<213> Artificial

<220>  
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53

<210> 17  
<211> 114  
<212> PRT  
<213> Artificial

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<220>

<223> Recombinantly produced 2325p6 protein occurring naturally in rana pipiens eggs and embryos.

<400> 17

Lys Pro Lys Glu Asp Lys Glu Trp Glu Lys Phe Lys Val Lys His Ile  
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Thr Ser Thr Met Asn Asn  
20 25 30

Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile  
35 40 45

His Ser Asn Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Ser Gly  
50 55 60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys Lys  
65 70 75 80

Asn Pro Lys Arg Cys Lys Tyr Ser Gln Ser Asn Glu Thr Asn Tyr Ile  
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile Gly  
100 105 110

Lys Cys

<210> 18

<211> 342

<212> DNA

<213> Artificial

<220>

<223> 2325p6 DNA occurring naturally in rana pipiens eggs and embryos.

<400> 18

aaaccgaaag aagacaaaga atggaaaaaa ttcaaaggta aacatatcac ttctcagtct 60

gttgctgact tcaactgcac ttctactatg aacaacccgg acttcactcc ggacggtag 120

tgcaaaccga tcaacacttt catccattct aacactggtc cggttaaaga aatctgccgt 180

cgtgcttctg gtcgtttaa caaatcttct actcagcagt tcccgctgac tacttgcaaa 240

aacccgaaac gttgcaaata ctctcagtct aacgaaacta actacatctg catcacttgc 300

cgtgacaact acccggttca tttcgtaaaa atcggtaaat gc 342

<210> 19

<211> 56

<212> DNA

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<213> Artificial

<220>

<223> SEQ ID NO:19

<400> 19

taattttgtt taacttaag aaggagatat accatgaaac cgaaagaaga caaaga

56

<210> 20

<211> 63

<212> DNA

<213> Artificial

<220>

<223> SEQ ID NO:20 Complementary to SEQ ID NO:19

<400> 20

ttcccattct ttgtttct tcgggtcat ggttatatctc cttttaaag ttaaacaaaa

60

tta

63

<210> 21

<211> 57

<212> DNA

<213> Artificial

<220>

<223> SEQ ID NO:21

<400> 21

atggaaaaaa ttcaaagtta aacatatcac ttctcagtct gttgctgact tcaactg

57

<210> 22

<211> 57

<212> DNA

<213> Artificial

<220>

<223> SEQ ID NO:22 Complementary to SEQ ID NO:21

<400> 22

agaagtgcag ttgaagtcag caacagactg agaagtgata tgtttaactt tgaattt

57

<210> 23

<211> 60

<212> DNA

<213> Artificial

<220>

<223> SEQ ID NO:23

<400> 23

cacttctact atgaacaacc cggacttcac tccggacggt cagtgcaaac cgatcaacac

60

<210> 24

<211> 60

<212> DNA

<213> Artificial

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<220>  
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<400> 24  
gatgaaagt gttgatcggtt tgcaactgacc gtccggagtg aagtccgggt tgttcatagt 60  
  
<210> 25  
<211> 52  
<212> DNA  
<213> Artificial  
  
<220>  
<223> SEQ ID NO:25  
  
<400> 25  
tttcatccat tctaacaactg gtccggtaa agaaatctgc cgtcgtgctt ct 52  
  
<210> 26  
<211> 52  
<212> DNA  
<213> Artificial  
  
<220>  
<223> SEQ ID NO:26 Complementary to SEQ ID NO:25  
  
<400> 26  
cacgaccaga agcacgacgg cagatttctt taaccggacc agtgttagaa tg 52  
  
<210> 27  
<211> 54  
<212> DNA  
<213> Artificial  
  
<220>  
<223> SEQ ID NO:27  
  
<400> 27  
ggtcgtgtta acaaatatcc tactcagcag ttcccgtga ctacttgcaa aaac 54  
  
<210> 28  
<211> 54  
<212> DNA  
<213> Artificial  
  
<220>  
<223> SEQ ID NO:28 Complement to SEQ ID NO:27  
  
<400> 28  
gtttcggtt tttgcaagta gtcagcggga actgctgagt agaagatttg ttaa 54  
  
<210> 29  
<211> 57  
<212> DNA  
<213> Artificial  
  
<220>  
<223> SEQ ID NO:29

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<400> 29  
ccgaaacgtt gcaaatactc tcagtctaac gaaactaact acatctgcac cacttgc 57

<210> 30  
<211> 57  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:30 Complement to SEQ ID NO:29

<400> 30  
tgtcacggca agtgatgcag atgttagtttttcgtttaga ctgagagtat ttgcaac 57

<210> 31  
<211> 60  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:31 Contains stop codon and BamHI site

<400> 31  
cgtgacaact acccggttca tttcgtaaaa atcggtaat gctagtaggg atccgcgcgg 60

<210> 32  
<211> 53  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:32 Complementary to SEQ ID NO:31

<400> 32  
ccgcgcggat ccctacttagc atttaccgat tttaacgaaa tgaaccgggt agt 53

<210> 33  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> SEQ ID NO:33 pET-11d Forward primer contains XbaI site

<400> 33  
caattccccct ctagaaataa ttttgtttaa cttaagaag gag 43

<210> 34  
<211> 114  
<212> PRT  
<213> Artificial

<220>  
<223> Recombinantly produced 2728 protein occurring naturally in rana pipiens eggs and embryos.

<400> 34

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Lys Pro Lys Glu Asp Lys Glu Trp Val Lys Phe Lys Ala Lys His Ile  
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Lys Thr Met Asn Asp  
20 25 30

Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe Ile  
35 40 45

His Ser Asn Thr Gly Pro Val Lys Asp Ile Cys Arg Arg Ala Ser Gly  
50 55 60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys Asn  
65 70 75 80

Lys Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile  
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile Gly  
100 105 110

Lys Cys

<210> 35  
<211> 342  
<212> DNA  
<213> Artificial

<220>  
<223> 2728 DNA occurring naturally in rana pipiens eggs and embryos.

<400> 35  
aaaccgaaag aagacaaaaga atgggttaaa ttcaaagcta aacatatac ttctcagtct 60  
gttgctgact tcaactgcaa caaaactatg aacgaccgg acttcactcc ggacggtcag 120  
tgcaaaccgg ttaacacttt catccattct aacactggtc cggttaaaga catctgccgt 180  
cgtgcttctg gtcgtgttaa caaatcttct actcagcagt tcccgctgac tacttgcaac 240  
aaaccgatcc gttgcaaata ctctcagtct aacactacta acttcatacg catcaacttg 300  
cgtgacaact acccggttca tttcgtaaaa atcggtaaat gc 342

<210> 36  
<211> 56  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:36

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<400> 36  
aattttgttt aactttaaga aggagatata catatgaaac cgaaagaaga caaaga 56

<210> 37  
<211> 56  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:37 Complement to SEQ ID NO:36

<400> 37  
aacccattct ttgtcttcct tcggttcat atgtatatct cttcttaaaa gttaaa 56

<210> 38  
<211> 56  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:38

<400> 38  
atgggttaaaa ttcaaagcta aacatatcac ttctcagtct gttgctgact tcaact 56

<210> 39  
<211> 56  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:39 Complement to SEQ ID NO:38

<400> 39  
tttgtgcagt tgaagtgcgc aacagactga gaagtgatat gtttagctt gaattt 56

<210> 40  
<211> 59  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:40

<400> 40  
gcaacaaaac tatgaacgac ccggacttca ctccggacgg tcagtcaaa ccggtaac 59

<210> 41  
<211> 59  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:41 Complementary to SEQ ID NO:40

<400> 41  
tgaaagtgtt aaccggttt cactgaccgt ccggagtgaa gtccgggtcg ttcatagtt 59

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<210> 42  
<211> 54  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:42

<400> 42  
actttcatcc attctaacac tggccgggtt aaagacatct gccgtcgtgc ttct 54

<210> 43  
<211> 54  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:43 Complementary to SEQ ID NO:42

<400> 43  
cacgaccaga agcacgacgg cagatgtctt taaccggacc agtgttagaa tgga 54

<210> 44  
<211> 54  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:44

<400> 44  
ggtcgtgtta acaaatcttc tactcagcag ttcccgctga ctacttgcaa caaa 54

<210> 45  
<211> 54  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:45 Complementary to SEQ ID NO:44

<400> 45  
ggatcgggtt gttgcaagta gtcagcggga actgctgagt agaagatttg ttaa 54

<210> 46  
<211> 57  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:46

<400> 46  
ccgatccgtt gcaaatactc tcagtctaac actactaact tcatctgcat cacttgc 57

<210> 47  
<211> 57

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<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:47 Complementary to SEQ ID NO:46

<400> 47  
tgtcacggca agtgatgcag atgaagtttag tagtgttaga ctgagagtat ttgcaac 57

<210> 48  
<211> 54  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:48

<400> 48  
cgtgacaact acccggttca tttcgtaaa atcggtaaat gctagtaggg atcc 54

<210> 49  
<211> 53  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:49 Complementary to SEQ ID NO:48

<400> 49  
ccgcgcggat ccctactagc atttaccgat tttaacgaaa tgaaccgggt agt 53

<210> 50  
<211> 42  
<212> DNA  
<213> Artificial

<220>  
<223> pET-22b Forward primer contains XbaI site

<400> 50  
gcccagccgg cgatggccaa accgaaagaa gaccgtgaat gg 42

<210> 51  
<211> 114  
<212> PRT  
<213> Artificial

<220>  
<223> Recombinantly produced 2325p4a protein occurring naturally in ran  
a pipiens eggs and embryos.

<400> 51

Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile  
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp  
20 25 30

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Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe Ile  
35 40 45

His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly  
50 55 60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys Lys  
65 70 75 80

Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile  
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly  
100 105 110

Lys Cys

<210> 52

<211> 342

<212> DNA

<213> Artificial

<220>

<223> 2325p4a DNA occurring naturally in rana pipiens eggs and embryos.

<400> 52

aaaccgaaag aagaccgtga atggaaaaaa ttcaaaaacta aacatatac ttctcagtct 60

gttgctgact tcaactgcaa ccgtactatg aacgaccgg cttacactcc ggacggtcag 120

tgc当地 accgg ttaacactt catccattct actactggc cggttaaaga aatctgccgt 180

cgtgctactg gtcgtgttaa caaatcttct actcagcagt tcactctgac tacttgaaa 240

aacccgatcc gttgcaaata ctctcagtct aacactacta acttcatctg catcaacttgc 300

cgtgacaact acccggttca tttcgtaaa actggtaat gc 342

<210> 53

<211> 39

<212> DNA

<213> Artificial

<220>

<223> SEQ ID NO:53 for substituting valine for isoleucine at position 4.  
4.

<400> 53

gacggtcagt gcaaaccggtaaacatttc atccattct 39

<210> 54

<211> 39

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<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:54 Complementary to SEQ ID NO:53

<400> 54  
agaatggatg aaagtgttaa ccggtttgca ctgaccgtc 39

<210> 55  
<211> 114  
<212> PRT  
<213> Artificial

<220>  
<223> "Cysteinized" protein.

<400> 55

Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile  
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp  
20 25 30

Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile  
35 40 45

His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly  
50 55 60

Arg Val Asn Lys Ser Ser Cys Gln Gln Phe Thr Leu Thr Thr Cys Lys  
65 70 75 80

Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile  
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly  
100 105 110

Lys Cys

<210> 56  
<211> 342  
<212> DNA  
<213> Artificial

<220>  
<223> DNA of "cysteinized" protein

<400> 56  
aaaccgaaag aagaccgtga atggaaaaaa ttcaaaaacta aacatatcac ttctcagtct 60

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gttgctgact tcaactgcaa ccgtactatg aacgaccgg cttacactcc ggacggtcag 120  
tgcaaaccga tcaacacttt catccattct actactggtc cggttaaaga aatctgccgt 180  
cgtgctactg gtcgtgttaa caaatcttct tgccagcagt tcactctgac tacttgcaaa 240  
aacccgatcc gttgcaaata ctctcagtct aacactacta acttcatctg catcaactgc 300  
cgtgacaact acccggttca tttcgtaaaa actggtaaat gc 342

<210> 57  
<211> 39  
<212> DNA  
<213> Artificial  
  
<220>  
<223> SEQ ID NO:57 Forward primer for substituting cysteine for threonine at position 71.  
  
<400> 57  
gttaacaaat cttcttgcca gcagttcact ctgactact 39

<210> 58  
<211> 39  
<212> DNA  
<213> Artificial  
  
<220>  
<223> SEQ ID NO:58 Reverse primer Complementary to SEQ ID NO:57  
  
<400> 58  
cagagtgaac tgctggcaag aagatttgtt aacacgacc 39

<210> 59  
<211> 115  
<212> PRT  
<213> Artificial  
  
<220>  
<223> Recombinantly produced 2325p4 protein with methionine in -1 position.  
  
<400> 59

Met Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His  
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn  
20 25 30

Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe  
35 40 45

Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr  
50 55 60

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Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys  
65 70 75 80

Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe  
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr  
100 105 110

Gly Lys Cys  
115

<210> 60

<211> 115

<212> PRT

<213> Artificial

<220>

<223> Recombinantly produced 2325p6 protein with methionine in -1 position.

<400> 60

Met Lys Pro Lys Glu Asp Lys Glu Trp Glu Lys Phe Lys Val Lys His  
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Thr Ser Thr Met Asn  
20 25 30

Asn Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe  
35 40 45

Ile His Ser Asn Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Ser  
50 55 60

Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys  
65 70 75 80

Lys Asn Pro Lys Arg Cys Lys Tyr Ser Gln Ser Asn Glu Thr Asn Tyr  
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile  
100 105 110

Gly Lys Cys  
115

<210> 61

<211> 115

<212> PRT

<213> Artificial

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<220>

<223> Recombinantly produced 2728 protein with methionine in -1 positio  
n.

<400> 61

Met Lys Pro Lys Glu Asp Lys Glu Trp Val Lys Phe Lys Ala Lys His  
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Lys Thr Met Asn  
20 25 30

Asp Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe  
35 40 45

Ile His Ser Asn Thr Gly Pro Val Lys Asp Ile Cys Arg Arg Ala Ser  
50 55 60

Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys  
65 70 75 80

Asn Lys Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe  
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile  
100 105 110

Gly Lys Cys  
115

<210> 62

<211> 2

<212> PRT

<213> Artificial

<220>

<223> 2 residues of a pelB leader sequence

<400> 62

Met Ala  
1

<210> 63

<211> 116

<212> PRT

<213> Artificial

<220>

<223> Recombinantly produced 2325p4 protein with pelB leader sequence t  
hat is 2 amino acid residues long.

<400> 63

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Met Ala Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys  
1 5 10 15

His Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met  
20 25 30

Asn Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr  
35 40 45

Phe Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala  
50 55 60

Thr Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr  
65 70 75 80

Cys Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn  
85 90 95

Phe Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys  
100 105 110

Thr Gly Lys Cys  
115

<210> 64

<211> 7

<212> PRT

<213> Artificial

<220>

<223> 7 residues of a pelB leader sequence

<400> 64

Ala Ala Gln Pro Ala Met Ala  
1 5

<210> 65

<211> 121

<212> PRT

<213> Artificial

<220>

<223> 2325p4 protein with pelB leader sequence that is 7 amino acid residues long.

<400> 65

Ala Ala Gln Pro Ala Met Ala Lys Pro Lys Glu Asp Arg Glu Trp Glu  
1 5 10 15

Lys Phe Lys Thr Lys His Ile Thr Ser Gln Ser Val Ala Asp Phe Asn

20

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25 30

Cys Asn Arg Thr Met Asn Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys  
35 40 45

Lys Pro Ile Asn Thr Phe Ile His Ser Thr Thr Gly Pro Val Lys Glu  
50 55 60

Ile Cys Arg Arg Ala Thr Gly Arg Val Asn Lys Ser Ser Thr Gln Gln  
65 70 75 80

Phe Thr Leu Thr Thr Cys Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln  
85 90 95

Ser Asn Thr Thr Asn Phe Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro  
100 105 110

Val His Phe Val Lys Thr Gly Lys Cys  
115 120

<210> 66

<211> 22

<212> PRT

<213> Artificial

<220>

<223> 22 residues of a pelB leader sequence

<400> 66

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala  
1 5 10 15

Ala Gln Pro Ala Met Ala  
20

<210> 67

<211> 136

<212> PRT

<213> Artificial

<220>

<223> Recombinantly produced 2325p4 protein with pelB leader sequence that is 22 amino acid residues long.

<400> 67

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala  
1 5 10 15

Ala Gln Pro Ala Met Ala Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys  
20 25 30

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Phe Lys Thr Lys His Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys  
35 40 45

Asn Arg Thr Met Asn Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys  
50 55 60

Pro Ile Asn Thr Phe Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile  
65 70 75 80

Cys Arg Arg Ala Thr Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe  
85 90 95

Thr Leu Thr Thr Cys Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser  
100 105 110

Asn Thr Thr Asn Phe Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val  
115 120 125

His Phe Val Lys Thr Gly Lys Cys  
130 135

<210> 68

<211> 115

<212> PRT

<213> Artificial

<220>

<223> Recombinantly produced 2325p4a protein with methionine in -1 position.

<400> 68

Met Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His  
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn  
20 25 30

Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe  
35 40 45

Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr  
50 55 60

Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys  
65 70 75 80

Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe  
85 90 95

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Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr  
100 105 110

Gly Lys Cys  
115

<210> 69

<211> 115

<212> PRT

<213> Artificial

<220>

<223> "Cysteinized" 2325p4-Cys71 protein with methionine in -1 position

<400> 69

Met Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His  
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn  
20 25 30

Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe  
35 40 45

Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr  
50 55 60

Gly Arg Val Asn Lys Ser Ser Cys Gln Gln Phe Thr Leu Thr Thr Cys  
65 70 75 80

Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe  
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr  
100 105 110

Gly Lys Cys  
115

<210> 70

<211> 176

<212> PRT

<213> Artificial

<220>

<223> "Cysteinized" fusion protein.

<400> 70

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His

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1

5

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10

15

Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn  
20 25 30

Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys  
35 40 45

Trp Trp Glu Leu Arg Gly Gly Ser Gly Gly Pro Gly Ser Lys Pro  
50 55 60

Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile Thr Ser  
65 70 75 80

Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp Pro Ala  
85 90 95

Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile His Ser  
100 105 110

Thr Thr Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly Arg Val  
115 120 125

Asn Lys Ser Ser Cys Gln Gln Phe Thr Leu Thr Thr Cys Lys Asn Pro  
130 135 140

Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile Cys Ile  
145 150 155 160

Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly Lys Cys  
165 170 175

<210> 71

<211> 528

<212> DNA

<213> Artificial

<220>

<223> DNA of "cysteinized" hEGF fusion protein.

<400> 71

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cgttgccagt accgtgacct gaaatggtgg gaaactgcgtg gtggttctgg tggtccgggt 180

ggttctaaac cgaaagaaga ccgtgaatgg gaaaaattca aaactaaaca tatcacttct 240

cagtctgttg ctgacttcaa ctgcaaccgt actatgaacg accccggctta cactccggac 300

ggtcagtgca aaccgatcaa cactttcatc cattctacta ctggtccgggt taaagaaatc 360

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tgccgtcgtg ctactggtcg tgttaacaaa tcttcttgcc agcagttcac tctgactact 420  
tgcaaaaacc cgatccgttg caaatactct cagtctaaca ctactaactt catctgcac 480  
acttgccgtg acaactaccc gtttcatttc gttaaaactg gttaaatgc 528

<210> 72  
<211> 55  
<212> DNA  
<213> Artificial

<220>  
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<210> 73  
<211> 54  
<212> DNA  
<213> Artificial

<220>  
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<400> 73  
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<210> 74  
<211> 54  
<212> DNA  
<213> Artificial

<220>  
<223> SEQ ID NO:74 EGF reverse primer

<400> 74  
agaaccaccc ggaccaccag aaccaccacg cagttcccac catttcaggt cacg 54